FILE 'CAPLUS' ENTERED AT 16:33:09 ON 16 SEP 2004

=> S INVASIVE CLEAVAGE
         20434 INVASIVE
             3 INVASIVES
         20436 INVASIVE
                 (INVASIVE OR INVASIVES)
        194477 CLEAVAGE
          5168 CLEAVAGES
        196926 CLEAVAGE
                 (CLEAVAGE OR CLEAVAGES)
L1   51 INVASIVE CLEAVAGE
                 (INVASIVE(W)CLEAVAGE)

=> S RNA;S DNA;S L1 AND (L2,L3)
        275944 RNA
         22023 RNAS
L2   279969 RNA
                 (RNA OR RNAS)

        679601 DNA
         17286 DNAS
L3   682258 DNA
                 (DNA OR DNAS)

L4   47 L1 AND ((L2 OR L3))

=> S (NON(W)TARGET)(4A)CLEAVAGE
        674039 NON
            33 NONS
        674065 NON
                 (NON OR NONS)
        264297 TARGET
         97323 TARGETS
        322533 TARGET
                 (TARGET OR TARGETS)
        194477 CLEAVAGE
          5168 CLEAVAGES
        196926 CLEAVAGE
                 (CLEAVAGE OR CLEAVAGES)
L5   13 (NON(W)TARGET)(4A)CLEAVAGE

=> S L4 AND L5
L6   6 L4 AND L5

=> S L1 NOT L6
L7   45 L1 NOT L6

=> D L6 1-6 CBIB ABS;D L7 1-45 TI

L6   ANSWER 1 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
2000:492042   Document No. 133:116707 **Invasive cleavage** of
     nucleic acids for detecting and characterizing target nucleic acids and
     microbial nucleases for the methods. Kaiser, Michael W.; Lyamichev,
     Victor I.; Lyamicheva, Natasha (Third Wave Technologies, Inc., USA). U.S.
     US 6090606 A 20000718, 262 pp., Cont.-in-part of U.S. Ser. No. 756,386.
     (English). CODEN: USXXAM. APPLICATION: US 1996-758314 19961202.
     PRIORITY: US 1996-599491 19960124; US 1996-682853 19960712; US 1996-756386

19961126; US 1996-756376 19961202.

AB Disclosed are methods for the detection and characterization of nucleic acid sequences and their variants by using structure-specific 5'-nucleases derived from thermostable **DNA** polymerases, e.g., of the FEN-1, RAD2, or XPG class of nucleases. The enzyme cleaves the target nucleic acid sequence at a structure formed via annealing with 2 pilot oligonucleotide sequences. Also disclosed are methods and devices for the separation of nucleic acid mols. based on charge. Also disclosed are methods for the detection of **non-target cleavage** products via the formation of a complete and activated protein binding region. Isolation of genes for endonuclease FEN-1 from Pyrococcus woesei and other microorganisms were described. Preparation of 5'-nucleases by deleting the C-terminal polymerase domain or by point mutations of Taq **DNA** polymerase was shown. The cleavage method was used for the identification of hepatitis C virus and human ras gene.

L6 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

1999:761460 Document No. 132:9599 Detection of nucleic acids by multiple sequential **invasive cleavages**. Hall, Jeff G.; Lyamichev, Victor I.; Mast, Andrea L.; Brow, Mary Ann D. (Third Wave Technologies, Inc., USA). U.S. US 5994069 A 19991130, 306 pp., Cont.-in-part of U.S. Ser. No. 759,038. (English). CODEN: USXXAM. APPLICATION: US 1997-823516 19970324. PRIORITY: US 1996-599491 19960124; US 1996-682853 19960712; US 1996-756386 19961126; US 1996-759038 19961202; US 1996-758314 19961202; WO 1997-US1072 19970122.

AB The present invention relates to means for the detection and characterization of nucleic acid sequences, as well as variations in nucleic acid sequences, by an Invader® oligonucleotide-directed cleavage detection assay. The present invention also relates to methods for forming a nucleic acid cleavage structure on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. The structure-specific nuclease activity of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. Derivs. of thermostable **DNA** polymerases and their mutants that retain their 5'-nuclease activity but lack polymerase activity are described for use in the nucleic acid detection system. The nuclease activity cleaves the single-stranded moiety of a Y-shaped structure and so is of use in selected cleavage of reporter sequences in a hybridization assay that includes 5'-nuclease-dependent cleavage and amplification steps. The present invention further relates to methods and devices for the separation of nucleic acid mols. based on charge. The present invention also provides methods for the detection of **non- target cleavage** products via the formation of a complete and activated protein binding region. The invention further provides sensitive and specific methods for the detection of human cytomegalovirus nucleic acid in a sample.

L6 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

1999:732986 Document No. 131:347456 **Invasive cleavage** of nucleic acids with thermostable 5'-nuclease for mutation detection and diagnostic applications.. Prudent, James R.; Hall, Jeff G.; Lyamichev, Victor I.; Brow, Mary Ann D.; Dahlberg, James E. (Third Wave Technologies, Inc., USA). U.S. US 5985557 A 19991116, 182 pp., Cont.-in-part of U.S. Ser. No. 682,853. (English). CODEN: USXXAM. APPLICATION: US 1996-756386 19961126. PRIORITY: US 1996-599491 19960124; US 1996-682853 19960712.

AB The present invention relates to means for the detection and characterization of nucleic acid sequences, as well as variations in nucleic acid sequences. The present invention also relates to methods for forming a nucleic acid cleavage structure on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. The structure-specific 5'-nuclease

activity of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. These 5'-nucleases are capable of cleaving linear duplex structures to create single discrete cleavage products identified using fluorescence imaging. The reaction involves a trigger and a detection reaction where a hairpin conformation is recognized. Here the target nucleic acid is not completely complementary to at least one of the first, second, third and fourth oligonucleotides. Assays where the target nucleic acid is reused or recycled during multiple rounds of hybridization with oligonucleotide probes and cleavage without the need to use temperature cyclin or nucleic acid synthesis. Through the interaction of the cleavage means an upstream oligonucleotide can be made to cleave a downstream oligonucleotide at an internal site in such a way that the resulting fragments of the downstream oligonucleotide dissociated from the target nucleic acid, thereby making that region of the target nucleic acid available for hybridization to another, uncleaved copy of the downstream oligonucleotide. The specific stability designed into the invader and probe sequences will depend on the temperature at which one desires to perform the reaction. It is desirable that the invader oligonucleotide be immediately available to direct the cleavage of each probe oligonucleotide that hybridizes to a target nucleic acid. For this reason, the invader oligonucleotide is provided in excess over the probe oligonucleotide. The **non-target cleavage** products are incubated with a template-independent polymerase and one nucleoside triphosphate under conditions such that at least one nucleotide is added to the 3'-hydroxyl group of the **non-target cleavage** products to generate tailed products. The present invention also provides novel methods and devices for the separation of nucleic acid mols. by charge by charge reversal. When an oligonucleotide is shortened through the action of a CLEAVASE enzyme or other cleavage agent, the pos. charge can be made to not only significantly reduce the net neg. charge, but to actually override it, effectively "flipping" the net charge of the labeled entity. The reversal of charge allows the products of target-specific cleavage to be partitioned from uncleaved probe by extremely simple means. It has clin. diagnostic applications as multiple alleles could be screened at once.

L6 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

1998:672692 Document No. 129:271498 Detection of nucleic acids and sequence variations by multiple sequential **invasive cleavages.**

Hall, Jeff G.; Lyamichev, Victor I.; Mast, Andrea L.; Brow, Mary Ann D.; Kwiatkowski, Robert W.; Vavra, Stephanie H. (Third Wave Technologies, Inc., USA). PCT Int. Appl. WO 9842873 A1 19981001, 524 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US5809 19980324. PRIORITY: US 1997-823516 19970324.

AB Claimed are methods for the detection and characterization of nucleic acid sequences by cleavage with multiple thermostable 5'-nucleases, the sequences of which are also claimed, for separation of nucleic acids based on charge, and for the detection of viral nucleic acids with these techniques. The method involves forming a nucleic acid cleavage structures by annealing oligonucleotides on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. The structure-specific nuclease activity of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The present invention further relates to methods and devices for the separation of nucleic acid mols. based on charge. The present invention also provides methods for the detection of **non-target cleavage** products via the formation of a complete and activated protein binding region. The invention further provides sensitive and specific methods for the detection of nucleic acid from various viruses in a sample.

L6 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
1998:388639 Document No. 129:64906 **Invasive cleavage** of nucleic acids for detecting and characterizing target nucleic acids and microbial nucleases for the methods. Kaiser, Michael W.; Lyamichev, Victor I.; Lyamicheva, Natasha (Third Wave Technologies, Inc., USA; Kaiser, Michael W.; Lyamichev, Victor I.; Lyamicheva, Natasha). PCT Int. Appl. WO 9823774 A1 19980604, 472 pp. DESIGNATED STATES: W: AU, CA, JP, US, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US21783 19971126. PRIORITY: US 1996-757653 19961129; US 1996-758314 19961202.

AB The present invention relates to means for the detection and characterization of nucleic acid sequences, as well as variations in nucleic acid sequences. The present invention also relates to improved cleavage means for the detection and characterization of nucleic acid sequences. Structure-specific nucleases derived from a variety of thermostable organisms are provided. These structure-specific nucleases are used to cleave target-dependent cleavage structures, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. Disclosed are methods for the detection and characterization of nucleic acid sequences and their variants by using structure-specific 5'-nucleases derived from thermostable **DNA** polymerases, or the FEN-1, RAD2, or XPG class of nucleases. The enzyme cleaves the target nucleic acid sequence at a structure formed via annealing with 2 pilot oligonucleotide sequences. Also disclosed are methods and devices for the separation of nucleic acid mols. based on charge. Also disclosed are methods for the detection of **non- target cleavage** products via the formation of a complete and activated protein binding region. Isolation of genes for endonuclease FEN-1 from Pyrococcus woesei, Methanococcus jannaschii, Archaeoglobus fulgidus, Methanobacterium thermoautotrophicum, and Pyrococcus furiosus are described. Preparation of 5'-nucleases by deleting the C-terminal polymerase domain or by point mutations of Taq **DNA** polymerase, and the preparation of chimeric enzymes of the FEN-1 endonucleases are also shown. The cleavage method was used for the identification of hepatitis C virus and human ras gene.

L6 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
1997:513643 Document No. 127:202064 **Invasive cleavage** of nucleic acids for detecting and characterizing target nucleic acids and microbial nucleases for the methods. Hall, Jeff G.; Lyamichev, Victor I.; Prudent, James R.; Brow, Mary Ann D.; Kaiser, Michael W.; Lyamichev, Natasha; Olive, David Michael; Dahlberg, James E.; et al. (Third Wave Technologies, Inc., USA; Hall, Jeff G.; Lyamichev, Victor I.; Prudent, James R.; Brow, Mary Ann D.; Kaiser, Michael W.; Lyamichev, Natasha; Olive, David Michael; Dahlberg, James E.). PCT Int. Appl. WO 9727214 A1 19970731, 456 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US1072 19970122. PRIORITY: US 1996-599491 19960124; US 1996-682853 19960712; US 1996-756386 19961126; US 1996-758314 19961202; US 1996-759038 19961202.

AB Disclosed are methods for the detection and characterization of nucleic acid sequences and their variants by using structure-specific 5'-nucleases derived from thermostable **DNA** polymerases, e.g., of the FEN-1, RAD2, or XPG class of nucleases. The enzyme cleaves the target nucleic acid sequence at a structure formed via annealing with 2 pilot oligonucleotide sequences. Also disclosed are methods and devices for the separation of nucleic acid mols. based on charge. Also disclosed are methods for the detection of **non-target cleavage** products via the formation of a complete and activated protein binding region. Isolation of genes for endonuclease FEN-1 from Pyrococcus woesei and other

microorganisms were described. Preparation of 5'-nucleases by deleting the C-terminal polymerase domain or by point mutations of Taq **DNA** polymerase was shown. The cleavage method was used for the identification of hepatitis C virus and human ras gene.

=> D 2,4-8,11,23,25,27-29,31-34,37-40,42-43,45 CBIB ABS

L7 ANSWER 2 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2004:597982 Surface **invasive cleavage** reactions for single nucleotide polymorphism analysis. Lu, Manchun (Univ. of Wisconsin, Madison, WI, USA). 144 pp. Avail. UMI, Order No. DA3101464 From: Diss. Abstr. Int., B 2004, 64(8), 3795 (English) 2003.
AB Unavailable

L7 ANSWER 4 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2004:453449 Document No. 141:34600 **Invasive cleavage** structure assays for detecting mutations in human gene CFTR associated with cystic fibrosis. Accola, Molly; Wigdal, Susan S.; Mast, Andrea L.; Bartholomay, Christian T.; Kwiatkowski, Robert W., Jr.; Tevere, Vincent; Ip, Hon S.; Carroll, Kathleen; Peterson, Patrick; Agarwal, Poonam; Jarvis, Nancy; Hall, Jeff G.; Heisler, Laura (Third Wave Technologies, Inc., USA). PCT Int. Appl. WO 2004046688 A2 20040603, 147 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US36611 20031114. PRIORITY: US 2002-PV426144 20021114; US 2003-371913 20030221; US 2003-606577 20030626; US 2003-PV489095 20030721; US 2003-PV497644 20030825; US 2003-PV515175 20031028; US 2003-713653 20031114.
AB The present invention provides compns. and methods for the detection and characterization of mutations associated with cystic fibrosis. More particularly, the present invention provides compns., methods and kits for using **invasive cleavage** structure assays (e.g. the INVADER assay) to screen nucleic acid samples from patients, for the presence of any one of a collection of mutations in the CFTR gene associated with cystic fibrosis. The present invention also provides compns., methods and kits for screening sets of CFTR alleles in a single reaction container. The invention related to detecting a set of CFTR alleles: (a) a first set comprising 2789+5G>A, R1162X, R560T, 1898+1G>A, delI507, I148T, and A455E; (b) a second set comprising 3120+1G>A, 3659delC, G551D, N1303K, 1078delT, R334W, 711+1G>T, and 3849+10 kb; (c) a third set comprising 621+1G>T, W1282X, 1717-1G>A, and R117H; (d) a fourth set comprising R347P, G85E, G542X, and R553X; and (e) a fifth set comprising 2184delA.

L7 ANSWER 5 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2004:412693 Document No. 140:418942 **Invasive cleavage** structure assays for detecting mutations in human gene CFTR associated with cystic fibrosis. Accola, Molly; Wigdal, Susan S.; Mast, Andrea L.; Bartholomay, Christian T.; Kwiatkowski, Robert W.; Tevere, Vincent; Ip, Hon S. (USA). U.S. Pat. Appl. Publ. US 2004096871 A1 20040520, 41 pp., Cont.-in-part of U.S. Ser. No. 371,913. (English). CODEN: USXXCO. APPLICATION: US 2003-606577 20030626. PRIORITY: US 2002-PV426144

20021114; US 2003-371913 20030221.

AB The present invention provides compns. and methods for the detection and characterization of mutations in human gene CFTR associated with cystic fibrosis. More particularly, the present invention provides compns., methods and kits for using **invasive cleavage** structure assays (e.g. the INVADER assay) to screen nucleic acid samples, e.g., from patients, for the presence of any one of a collection of mutations in the CFTR gene associated with cystic fibrosis. The present invention also provides probes and kits for screening sets of CFTR alleles in a single reaction container. The invention related to detecting a set of CFTR alleles: (a) a first set comprising 2789+5G>A, R1162X, R560T, 1898+1G>A, delI507, I148T, and A455E; (b) a second set comprising 3120+1G>A, 3659delC, G551D, N1303K, 1078delT, R334W, 711+1G>T, and 3849+10 kb; (c) a third set comprising 621+1G>T, W1282X, 1717-1G>A, and R117H; (d) a fourth set comprising R347P, G85E, G542X, and R553X; and (e) a fifth set comprising 2184delA.

L7 ANSWER 6 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2004:412688 Document No. 140:418941 **Invasive cleavage** structure assays for detecting mutations in human gene CFTR associated with cystic fibrosis. Accola, Molly; Wigdal, Susan S.; Mast, Andrea L.; Bartholomay, Christian T.; Kwiatkowski, Robert W.; Tevere, Vincent; Ip, Hon S. (USA). U.S. Pat. Appl. Publ. US 2004096844 A1 20040520, 40 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-371913 20030221. PRIORITY: US 2002-PV426144 20021114.

AB The present invention provides compns. and methods for the detection and characterization of mutations in human gene CFTR associated with cystic fibrosis. More particularly, the present invention provides compns., methods and kits for using **invasive cleavage** structure assays (e.g. the INVADER assay) to screen nucleic acid samples, e.g., from patients, for the presence of any one of a collection of mutations in the CFTR gene associated with cystic fibrosis. The present invention also provides probes and kits for screening sets of CFTR alleles in a single reaction container. The invention related to detecting a set of CFTR alleles: (a) a first set comprising 2789+5G>A, R1162X, R560T, 1898+1G>A, delI507, I148T, and A455E; (b) a second set comprising 3120+1G>A, 3659delC, G551D, N1303K, 1078delT, R334W, 711+1G>T, and 3849+10 kb; (c) a third set comprising 621+1G>T, W1282X, 1717-1G>A, and R117H; (d) a fourth set comprising R347P, G85E, G542X, and R553X; and (e) a fifth set comprising 2184delA.

L7 ANSWER 7 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2004:320657 Document No. 141:18270 **Invasive cleavage** reactions on DNA-modified diamond surfaces. Lu, Manchun; Knickerbocker, Tanya; Cai, Wei; Yang, Wensha; Hamers, Robert J.; Smith, Lloyd M. (Department of Chemistry, University of Wisconsin-Madison, Madison, WI, 53706-1396, USA). Biopolymers, 73(5), 606-613 (English) 2004. CODEN: BIPMAA. ISSN: 0006-3525. Publisher: John Wiley & Sons, Inc..

AB Recently developed DNA-modified diamond surfaces exhibit excellent chemical stability to high-temperature incubations in biol. buffers. The stability of these surfaces is substantially greater than that of gold or silicon surfaces, using similar surface attachment chemical The DNA mols. attached to the diamond surfaces are accessible to enzymes and can be modified in surface enzymic reactions. An important application of these surfaces is for surface **invasive cleavage** reactions, in which target DNA strands added to the solution may result in specific cleavage of surface-bound probe oligonucleotides, permitting anal. of single nucleotide polymorphisms (SNPs). Our previous work demonstrated the feasibility of performing such cleavage reactions on planar gold surfaces using PCR-amplified human genomic DNA as target. The sensitivity of detection in this earlier work was substantially limited by a

lack of stability of the gold surface employed. In the present work, detection sensitivity is improved by a factor of .apprx.100 (100 amole of DNA target compared with 10 fmole in the earlier work) by replacing the DNA-modified gold surface with a more stable DNA-modified diamond surface.

L7 ANSWER 8 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2004:310744 Document No. 140:333521 Detection of specific nucleic acid sequences using INVADER oligonucleotide-directed cleavage using thermostable derivatives of DNA polymerases with thermostable 5'-nuclease activities. Lyamichev, Victor; Neri, Bruce P.; Hall, Jeff; Lukowiak, Andrew (USA). U.S. Pat. Appl. Publ. US 2004072182 A1 20040415, 393 pp., Cont.-in-part of U.S. Pat. Appl. 2003 152,971. (English). CODEN: USXXCO. APPLICATION: US 2003-356861 20030203. PRIORITY: US 1996-756386 19961126; WO 1997-US1072 19970122; WO 1998-US5809 19980324; US 1999-350309 19990709; US 2000-713601 20001115; US 2001-PV344946 20011107; US 2002-PV361060 20020227; US 2002-290386 20021107.

AB The present invention relates to compns. and methods for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences by the formation of specific cleavage structures. A 5' nuclease activity from any of a variety of enzymes is used to cleave the target-dependent cleavage structure, with the release of the cleavage product indicating the presence of the target sequences. The cleavage agents and polymerases find use, for example, for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. A sample suspected of containing the target sequence is contacted with oligonucleotides capable of forming an **invasive cleavage** structure in the present of the target sequence and with an agent for detecting the presence of the **invasive cleavage** structure. ARRESTOR oligonucleotides improve sensitivity of multiple sequential **invasive cleavage** assays and allow use of higher concns. of primary probe without increasing background signal. The detailed description of the invention includes: improved enzymes for the use in INVADER oligonucleotide-directed cleavage reactions comprising RNA targets; reaction design for INVADER assay detection of RNA targets; kits for performing the RNA invader assay; and the INVADER assay for direct detection and measurement of specific RNA analytes.

L7 ANSWER 11 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2004:78618 Document No. 140:140615 RNA target detection using INVADER oligonucleotide-directed cleavage using thermostable derivatives of DNA polymerases with thermostable 5'-nuclease activities. Ma, Wupo; Lyamichev, Victor; Kaiser, Michael; Lyamichieva, Natalie E.; Allawi, Hatin Taysir; Lukowiak, Andrew A.; Schaefer, James J.; Lukowiak, Andrew A. (USA). U.S. Pat. Appl. Publ. US 2004018489 A1 20040129, 250 pp., Cont.-in-part of U.S. Ser. No. 758,282. (English). CODEN: USXXCO. APPLICATION: US 2001-864426 20010524. PRIORITY: US 1996-599491 19960124; US 1996-682853 19960712; US 1996-756386 19961126; US 1996-759038 19961202; WO 1997-US1072 19970122; US 1997-823516 19970324; US 1999-350309 19990709; US 2000-381212 20000208; US 2000-577304 20000524; US 2001-758282 20010111.

AB The present invention relates to compns. and methods for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. The present invention relates to methods for forming a nucleic acid cleavage structure on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. For example, in some embodiments, a 5' nuclease activity from any of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The present invention provides novel cleavage agents and polymerases for the cleavage and modification of nucleic acid. The cleavage agents and

polymerases find use, for example, for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. In some embodiments, the 5'-nuclease activity of a variety of modified Thermus polymerase enzymes is used to cleave a target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The term "cleavage structure" refers to a structure that is formed by the interaction of at least one probe oligonucleotide (called the INVADER oligonucleotide) and a target nucleic acid, forming a structure comprising a duplex, the resulting structure being cleavable by a cleavage agent including but not limited to an enzyme. A sample suspected of containing the target sequence is contacted with oligonucleotides capable of forming an **invasive cleavage** structure in the present of the target sequence and with an agent for detecting the presence of the **invasive cleavage** structure. ARRESTOR oligonucleotides improve sensitivity of multiple sequential **invasive cleavage** assays and allow use of higher concns. of primary probe without increasing background signal. The detailed description of the invention includes: (1) improved enzymes for the use in INVADER oligonucleotide-directed cleavage reactions comprising RNA targets; (2) reaction design for INVADER assay detection of RNA targets; (3) kits for performing the RNA invader assay; and (4) the INVADER assay for direct detection and measurement of specific RNA analytes.

L7 ANSWER 23 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2003:221045 Document No. 140:71640 Improved sensitivity for solid-support **invasive cleavage** reactions with flow cytometry analysis. [Erratum to document cited in CA138:249257]. Stevens, P. Wilkins; Rao, K. V. N.; Hall, J. G.; Lyamichev, V.; Neri, B. P.; Kelso, D. M. (Northwestern University, Evanston, IL, 60208-3107, USA). BioTechniques, 34(3), 496 (English) 2003. CODEN: BTNQDO. ISSN: 0736-6205. Publisher: Eaton Publishing Co..
AB The article was incorrectly labeled "Short Tech. Report" on the title page; it is a Product Application Focus.

L7 ANSWER 25 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2003:43824 Document No. 138:249257 Improved sensitivity for solid-support **invasive cleavage** reactions with flow cytometry analysis. Stevens, P. Wilkins; Rao, K. V. N.; Hall, J. G.; Lyamichev, V.; Neri, B. P.; Kelso, D. M. (Northwestern University, Evanston, IL, 60208-3107, USA). BioTechniques, 34(1), 198-203 (English) 2003. CODEN: BTNQDO. ISSN: 0736-6205. Publisher: Eaton Publishing Co..
AB A new configuration of the solid-support **invasive cleavage** reaction provides a small reaction-volume format for high-sensitivity discrimination of nucleic acid targets with single nucleotide differences. With target concns. as low as 2 amol/assay, the solid-support **invasive cleavage** reaction clearly distinguishes single base mutations. Two oligonucleotides tethered to the solid support hybridize to the target nucleic acid, forming a tripartite substrate that can be recognized and cleaved by Cleavase, a structure-specific 5'-nuclease. Each cleavage event yields fluorescence signal on the surface. When microspheres serve as the solid-support surface, anal. by fluorometer imparts real-time information about change in the reaction signal over time. Flow cytometry provides an alternative detection technol. that collects endpoint information about the reaction signal on individual microspheres. A reaction volume of 10 μL with as few as 3000 microspheres is sufficient to distinguish single nucleotide differences at target concns. less than 200 fM. This sensitivity level is within the range required for anal. of SNPs in genomic DNA. In addition, the flow cytometry format has multiplexing potential, making the microsphere-based **invasive cleavage** assay attractive for high-throughput genomic applications.

L7 ANSWER 27 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2002:696174 Document No. 137:227596 Detection of DNA and RNA targets using INVADER oligonucleotide-directed cleavage reactions using modified Thermus polymerase enzymes with thermostable 5'-nuclease activities and FEN-1 endonucleases. Lyamichev, Victor I.; Kaiser, Michael W.; Lyamicheva, Natasha (Third Wave Technologies, Inc., USA). PCT Int. Appl. WO 2002070755 A2 20020912, 871 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US44953 20011115. PRIORITY: US 2000-713601 20001115; US 2000-714935 20001117.

AB The present invention provides novel cleavage agents and polymerases for the cleavage and modification of nucleic acids. The cleavage agents and polymerases find use, for example, for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. In some embodiments, the 5'-nuclease activity of a variety of modified Thermus polymerase enzymes is used to cleave a target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The term "cleavage structure" refers to a structure that is formed by the interaction of at least one probe oligonucleotide (called the INVADER oligonucleotide) and a target nucleic acid, forming a structure comprising a duplex, the resulting structure being cleavable by a cleavage agent including but not limited to an enzyme. A sample suspected of containing the target sequence is contacted with oligonucleotides capable of forming an **invasive cleavage** structure in the present of the target sequence and with an agent for detecting the presence of the **invasive cleavage** structure. ARRESTOR oligonucleotides improve sensitivity of multiple sequential **invasive cleavage** assays and allow use of higher concns. of primary probe without increasing background signal. The detailed description of the invention includes: (1) detection of specific nucleic acid sequences using 5'-nucleases in an INVADER-directed cleavage assay; (2) signal enhancement by incorporating the products of an **invasive cleavage** reaction into a subsequent **invasive cleavage** reaction; (3) effect of ARRESTOR oligonucleotides on signal and background in sequential **invasive cleavage** reactions; (4) improved enzymes for the use in INVADER oligonucleotide-directed cleavage reactions comprising RNA targets; (5) reaction design for INVADER assay detection of RNA targets; (6) kits for performing the RNA invader assay; and (7) the INVADER assay for direct detection and measurement of specific RNA analytes. FEN-1 endonucleases (and their modified forms) from a variety of microbial sources are also characterized and shown to be effective in INVADER-directed cleavage assays.

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2002:531606 Document No. 137:74482 Detection of genetic polymorphisms in drug-metabolizing enzyme genes and their use for evaluation and screening of drugs. Nakamura, Yusuke; Sekine, Akihiro; Iida, Aritoshi; Saito, Susumu (Riken Corp., Japan). PCT Int. Appl. WO 2002052044 A2 20020704, 2858 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,

VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-XA11592 20011227. PRIORITY: JP 2000-399443 20001227; JP 2001-135256 20010502; JP 2001-256862 20010827; WO 2001-JP11592 20011227.

AB The present invention relates to genetic polymorphism data, compns. and methods for detecting genetic polymorphisms, methods for evaluating drugs using genetic polymorphisms, and screening methods for drugs. Thus, 7669 sep. single nucleotide polymorphisms (SNP) are provided in human genes encoding drug-metabolizing enzymes. In some embodiments, a drug-metabolizing enzyme is at least one of the following: epoxide hydrolase, methyltransferase, N-acetyltransferase, sulfotransferase, quinone oxidoreductase, glutathione S-transferase, UDP- glycosyltransferase, aldehyde dehydrogenase, alc. dehydrogenase, esterase, NDUF, cytochrome P 450, and ATP-binding cassette. In one example, a correlation is demonstrated between optimal amts. of azathioprine (an immunosuppressive agent) and various combinations of the alleles at the 868th SNP of intron 3 of thiopurine S-methyltransferase gene (G or T alleles) and the 2682nd SNP of intron 3 (C or A alleles). [This abstract record is one of two records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L7 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2002:504959 Document No. 137:74459 Detection of genetic polymorphisms in drug-metabolizing enzyme genes and their use for evaluation and screening of drugs. Nakamura, Yusuke; Sekine, Akihiro; Iida, Aritoshi; Saito, Susumu (Riken Corp., Japan). PCT Int. Appl. WO 2002052044 A2 20020704, 2858 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-JP11592 20011227. PRIORITY: JP 2000-399443 20001227; JP 2001-135256 20010502; JP 2001-256862 20010827.

AB The present invention relates to genetic polymorphism data, compns. and methods for detecting genetic polymorphisms, methods for evaluating drugs using genetic polymorphisms, and screening methods for drugs. Thus, 7669 sep. single nucleotide polymorphisms (SNP) are provided in human genes encoding drug-metabolizing enzymes. In some embodiments, a drug-metabolizing enzyme is at least one of the following: epoxide hydrolase, methyltransferase, N-acetyltransferase, sulfotransferase, quinone oxidoreductase, glutathione S-transferase, UDP- glycosyltransferase, aldehyde dehydrogenase, alc. dehydrogenase, esterase, NDUF, cytochrome P 450, and ATP-binding cassette. In one example, a correlation is demonstrated between optimal amts. of azathioprine (an immunosuppressive agent) and various combinations of the alleles at the 868th SNP of intron 3 of thiopurine S-methyltransferase gene (G or T alleles) and the 2682nd SNP of intron 3 (C or A alleles). [This abstract record is one of two records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L7 ANSWER 31 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2002:452271 Document No. 137:42261 Structure-specific DNA cleavage on surfaces. Lu, Manchun; Hall, Jeff G.; Shortreed, Michael R.; Wang, Liman;

Berggren, W. Travis; Stevens, Priscilla Wilkins; Kelso, David M.; Lyamichev, Victor; Neri, Bruce; Skinner, James L.; Smith, Lloyd M. (Department of Chemistry, University of Wisconsin-Madison, Madison, WI, 53706-1396, USA). Journal of the American Chemical Society, 124(27), 7924-7931 (English) 2002. CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.

AB The structure-specific **invasive cleavage** reaction is a useful means for sensitive and specific detection of single nucleotide polymorphisms, or SNPs, directly from genomic DNA without a need for prior target amplification. A new approach integrating this **invasive cleavage** assay and surface DNA array technol. has been developed for potentially large-scale SNP scoring in a parallel format. Two surface **invasive cleavage** reaction strategies were designed and implemented for a model SNP system in codon 158 of the human ApoE gene. The upstream oligonucleotide, which is required for the **invasive cleavage** reaction, is either co-immobilized on the surface along with the probe oligonucleotide or alternatively added in solution The ability of this approach to unambiguously discriminate a single base difference was demonstrated using PCR-amplified human genomic DNA. A theor. model relating the surface fluorescence intensity to the progress of the **invasive cleavage** reaction was developed and agreed well with exptl. results.

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2002:369431 Document No. 138:20035 A surface **invasive cleavage** assay for highly parallel SNP analysis. Lu, Manchun; Shortreed, Michael R.; Hall, Jeff G.; Wang, Liman; Berggren, Travis; Stevens, Priscilla Wilkins; Kelso, David M.; Lyamichev, Victor; Neri, Bruce; Smith, Lloyd M. (Department of Chemistry, University of Wisconsin-Madison, Madison, WI, 53706, USA). Human Mutation, 19(4), 416-422 (English) 2002. CODEN: HUMUE3. ISSN: 1059-7794. Publisher: Wiley-Liss, Inc..

AB The structure-specific **invasive cleavage** of single-stranded DNA by 5' nucleases is a useful means for sensitive detection of single-nucleotide polymorphisms or SNPs. The solution-phase **invasive cleavage** reaction has sufficient sensitivity for direct detection of as few as 600 target mols. with no prior target amplification. One approach to the parallelization of SNP anal. is to adapt the **invasive cleavage** reaction to an addressed array format. Two surface **invasive cleavage** reaction strategies were designed and tested using the polymorphic site in codon 158 of the human ApoE gene as a model system, with a synthetic oligonucleotide as target. The upstream oligonucleotide, which is required for the **invasive cleavage** reaction, was either added in solution (strategy 1), or co-immobilized on the surface along with the probe oligonucleotide (strategy 2). Both strategies showed target-concentration and time-dependent amplification of signal. Parameters that govern the rate of the surface-**invasive cleavage** reactions are discussed.

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2002:183940 Document No. 136:211421 Multiplexed gene expression analysis using the invader RNA assay with MALDI-TOF mass spectrometry detection. Berggren, W. Travis; Takova, Tsetska; Olson, Marilyn C.; Eis, Peggy S.; Kwiatkowski, Robert W.; Smith, Lloyd M. (Department of Chemistry, University of Wisconsin, Madison, WI, 53706, USA). Analytical Chemistry, 74(8), 1745-1750 (English) 2002. CODEN: ANCHAM. ISSN: 0003-2700. Publisher: American Chemical Society.

AB A mass spectrometric approach for measuring gene expression levels has been developed. This technique utilizes a signal amplification system and anal. by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Signal amplification from the targeted RNA employs a recently developed **invasive cleavage** assay that does not require prior PCR

amplification. The assay uses a set of target-specific probes (oligonucleotides), which hybridize to the RNA being measured to create an overlap structure with a single-stranded flap. This flap is enzymically cleaved and accumulates linearly in a target-specific manner. The products of the reaction, short DNA oligomers, are well suited for quant. detection by MALDI-TOF mass spectrometry. Multiplexing is achieved by designing the assays so that reaction products for different mRNA targets have discrete masses that can be resolved in a single mass spectrum. Simultaneous anal. of human cytokine in vitro transcripts IL-1β, TNF-α, and IL-6, with GAPDH as a reference standard, was used as a model system to demonstrate this novel method of gene expression anal.

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2002:181111 Document No. 139:79646 An **invasive cleavage** assay for direct quantitation of specific RNAs. [Erratum to document cited in CA136:145705]. Eis, Peggy S.; Olson, Marilyn C.; Takova, Tsetska; Curtis, Michelle L.; Olson, Sarah M.; Vener, Tatiana I.; Ip, Hon S.; Vedvik, Kevin L.; Bartholomay, Christian T.; Allawi, Hatim T.; Ma, Wu-Po; Hall, Jeff G.; Morin, Michelle D.; Rushmore, Tom H.; Lyamichev, Victor I.; Kwiatkowski, Robert W. (Third Wave Technologies, Madison, WI, 53719-1256, USA). Nature Biotechnology, 20(3), 307 (English) 2002. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature America Inc..

AB The lysis buffer mentioned in the Figure 4 legend was described incorrectly as containing "200 mg/mL tRNA". The correct sentence is as follows: "Cell lysate preparation: 40 μl lysis buffer (20 mM Tris, pH 8, 5 mM MgCl2, 0.5% NP-40, 20 μg/mL tRNA) was added per well, cells were lysed at room temperature for 3-5 min, 30 μl of each lysate sample were transferred to a 96-well microplate and cellular nucleases inactivated by heating the lysates for 15 min at 75-80°C.".

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2001:868661 Document No. 136:49292 Detection of RNA targets using INVADER oligonucleotide-directed cleavage reactions and construction of modified Thermus polymerase enzymes with thermostable 5'-nuclease activities. Allawi, Hatim; Bartholomay, Christian Tor; Chehak, Luanne; Curtis, Michelle L.; Eis, Peggy S.; Hall, Jeff G.; Ip, Hon S.; Kaiser, Michael; Kwiatkowski, Robert W., Jr.; Lukowiak, Andrew A.; Lyamichev, Victor; Ma, Wupo; Olson-munoz, Marilyn C.; Olson, Sarah M.; Schaefer, James J.; Skrzypczynski, Zbigniew; Takova, Tsetska Y.; Vedvik, Kevin L.; Lyamichev, Natalie E.; Neri, Bruce P. (Third Wave Technologies, Inc., USA). PCT Int. Appl. WO 2001090337 A2 20011129, 1266 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US17086 20010524. PRIORITY: US 2000-577304 20000524; US 2001-758282 20010111; US 2001-864426 20010524; US 2001-864636 20010524.

AB The present invention provides novel cleavage agents and polymerases for the cleavage and modification of nucleic acid. The cleavage agents and polymerases find use, for example, for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. In some embodiments, the 5'-nuclease activity of a variety of modified Thermus polymerase enzymes is used to cleave a target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific

variations thereof. The term "cleavage structure" refers to a structure that is formed by the interaction of at least one probe oligonucleotide (called the INVADER oligonucleotide) and a target nucleic acid, forming a structure comprising a duplex, the resulting structure being cleavable by a cleavage agent including but not limited to an enzyme. A sample suspected of containing the target sequence is contacted with oligonucleotides capable of forming an **invasive cleavage** structure in the present of the target sequence and with an agent for detecting the presence of the **invasive cleavage** structure. ARRESTOR oligonucleotides improve sensitivity of multiple sequential **invasive cleavage** assays and allow use of higher concns. of primary probe without increasing background signal. The detailed description of the invention includes: (1) detection of specific nucleic acid sequences using 5'-nucleases in an INVADER-directed cleavage assay; (2) signal enhancement by incorporating the products of an **invasive cleavage** reaction into a subsequent **invasive cleavage** reaction; (3) effect of ARRESTOR oligonucleotides on signal and background in sequential **invasive cleavage** reactions; (4) improved enzymes for the use in INVADER oligonucleotide-directed cleavage reactions comprising RNA targets; (5) reaction design for INVADER assay detection of RNA targets; (6) kits for performing the RNA invader assay; and (7) the INVADER assay for direct detection and measurement of specific RNA analytes.

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2001:651626 Document No. 136:211409 Analysis of single nucleotide polymorphisms with solid phase **invasive cleavage** reactions. Stevens, Priscilla Wilkins; Hall, Jeff G.; Lyamichev, Victor; Neri, Bruce P.; Lu, Manchun; Wang, Liman; Smith, Lloyd M.; Kelso, David M. (Department of Biomedical Engineering, Robert R. McCormick School of Engineering and Applied Science, Northwestern University, Evanston, IL, 60208-3107, USA). Nucleic Acids Research, 29(16), e77/1-e77/8 (English) 2001. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB Using microparticles as the capture surface and fluorescence resonance energy transfer as the detection technol., we have demonstrated the feasibility of performing the **invasive cleavage** reaction on a solid phase. An effective tool for many genomic applications, the solution phase **invasive cleavage** assay is a signal, amplification method capable of distinguishing nucleic acids that differ by only a single base mutation. The method positions two overlapping oligonucleotides, the probe and upstream oligonucleotides, on the target nucleic acid to create a complex recognized and cleaved by a structure-specific 5'-nuclease. For microarray and other multiplex applications, however, the method must be adapted to a solid phase platform. Effective cleavage of the probe oligonucleotide occurred when either of the two required overlapping oligonucleotides was configured as the particle-bound reagent and also when both oligonucleotides were attached to the solid phase. Positioning probe oligonucleotides away from the particle surface via long tethers improved both the signal and the reaction rates. The particle-based **invasive cleavage** reaction was capable of distinguishing the ApoE Cys158 and Arg158 alleles at target concns. as low as 100 amol/assay (0.5 pM).

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2001:621293 Document No. 136:227420 Cytochrome P450 induction in rat hepatocytes assessed by quantitative real-time reverse-transcription polymerase chain reaction and the RNA **invasive cleavage** assay. Burczynski, Michael E.; McMillian, Michael; Parker, James B.; Bryant, Stewart; Leone, Angelique; Grant, Elfrida R.; Thorne, Jacqueline M.; Zhong, Zhong; Zivin, Robert A.; Johnson, Mark D. (Departments of Pre-Clinical Drug Metabolism and Toxicology, The R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ, 08869, USA). Drug

Metabolism and Disposition, 29(9), 1243-1250 (English) 2001. CODEN: DMDSAI. ISSN: 0090-9556. Publisher: American Society for Pharmacology and Experimental Therapeutics.

AB The acceleration of drug discovery due to combinatorial chemical and high-throughput screening methods has increased the nos. of candidate pharmaceuticals entering the drug development phase, and the capability to accurately predict whether drug candidates will induce various members of the drug-metabolizing cytochrome P 450 (CYP) enzyme superfamily is currently of great interest to the pharmaceutical industry. In the present study, we describe the rapid and reliable anal. of CYP induction in a readily obtained model system (cultured rat hepatocytes) using both real-time quant. reverse transcription-polymerase chain reaction (real-time RT-PCR) and the RNA **invasive cleavage** assay. The levels of members in the three primary inducible rat CYP subfamilies (CYP1A1, CYP2B1/2, and CYP3A1) were analyzed in untreated and induced (β-naphthoflavone, phenobarbital, and hydrocortisone) hepatocyte cultures under various media conditions to screen for optimal CYP induction profiles. The fold inductions measured by real-time RT-PCR and the RNA **invasive cleavage** assay were also compared with enzyme activity measurements in parallel cultures using liquid chromatog./double mass spectrometry-based assays, and the sensitivity and the specificity of the two RNA anal. methods were compared. Using these techniques, various culture conditions were examined for optimizing induction of the three CYP subfamily members. Both real-time RT-PCR and the RNA **invasive cleavage** assay prove to be effective methods for determining the effects of drugs on specific CYPs in primary rat hepatocytes.

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2001:495829 Document No. 136:145705 An **invasive cleavage** assay for direct quantitation of specific RNAs. Eis, Peggy S.; Olson, Marilyn C.; Takova, Tsetska; Curtis, Michelle L.; Olson, Sarah M.; Vener, Tatiana I.; Ip, Hon S.; Vedvik, Kevin L.; Bartholomay, Christian T.; Allawi, Hatim T.; Ma, Wu-Po; Hall, Jeff G.; Morin, Michelle D.; Rushmore, Tom H.; Lyamichev, Victor I.; Kwiatkowski, Robert W. (Third Wave Technologies, Madison, WI, 53719-1256, USA). Nature Biotechnology, 19(7), 673-676 (English) 2001. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature America Inc..

AB RNA quantitation is becoming increasingly important in basic, pharmaceutical, and clin. research. For example, quantitation of viral RNAs can predict disease progression and therapeutic efficacy. Likewise, gene expression anal. of diseased vs. normal, or untreated vs. treaded, tissue can identify relevant biol. responses or assess the effects of pharmacol. agents. As the focus of the Human Genome Project moves toward gene expression anal., the field will require a flexible RNA anal. technol. that can quant. monitor multiple forms of alternatively transcribed and/or processed RNAs. We have applied the principles of **invasive cleavage** and engineered an improved 5'-nuclease to develop an isothermal, fluorescence resonance energy transfer (FRET)-based signal amplification method for detecting RNA in both total RNA and cell lysate samples. This detection format, termed the RNA **invasive cleavage** assay, obviates the need for target amplification or addnl. enzymic signal enhancement. In this report, we describe the assay and present data demonstrating its capabilities for sensitive (<100 copies per reaction), specific (discrimination of 95% homologous sequences, 1 in ≥20,000), and quant. (1,2-fold changes in RNA levels) detection of unamplified RNA in both single- and biplex-reaction formats.

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2000:591746 Document No. 134:51975 Genotyping of factor V G1691A (Leiden) without the use of PCR by **invasive cleavage** of

oligonucleotide probes. Hessner, Martin J.; Budish, Mary Ann; Friedman, Kenneth D. (The Diagnostic Laboratories of the Blood Center of Southeastern Wisconsin, Milwaukee, WI, 53201-2178, USA). Clinical Chemistry (Washington, D. C.), 46(8, Pt. 1), 1051-1056 (English) 2000. CODEN: CLCHAU. ISSN: 0009-9147. Publisher: American Association for Clinical Chemistry.

AB Background: The factor V G1691A Leiden (FVL) mutation is the most common known hereditary risk factor for venous thrombosis. Methods: Third Wave Technologies, Inc. (Madison, WI) has developed a new microtiter plate-based assay that does not require PCR, restriction digestion, or gel electrophoresis. This technol. system, termed the Invader assay, utilizes a 5' "invading" oligonucleotide, and a partially overlapping 3' "signal" oligonucleotide, which together form a specific structure when bound to a complementary genomic DNA template. A thermostable flap endonuclease cleaves this structure, releasing the 5' flap from the signal oligonucleotide. Increased temperature and an excess of the signal probe enable multiple probes to be cleaved for each target sequence present without temperature cycling. The cleaved probes then direct cleavage of a secondary probe, which is 5' end-labeled with fluorescein but is quenched by an internal dye. Upon cleavage, the fluorescein-labeled product is detected using a standard fluorescence plate reader. Genotypes are determined by net wild-type/mutant signal ratio. Results: Complete concordance was observed, after resolution of four discordances, when 1369 individuals (1264 wild type, 102 heterozygous, 3 homozygous) were FVL genotyped by both the Invader assay and by allele-specific PCR. Conclusion: the authors conclude that FVL genotyping using **invasive cleavage** of oligonucleotide probes is a rapid and reliable alternative to genotyping by more traditional PCR-based methods.

L7 ANSWER 43 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2000:384537 Document No. 134:142431 A multi-site study for detection of the factor V (Leiden) mutation from genomic DNA using a homogeneous invader microtiter plate fluorescence resonance energy transfer (FRET) assay. Ledford, Marlies; Friedman, Kenneth D.; Hessner, Martin J.; Moehlenkamp, Cynthia; Williams, Thomas M.; Larson, Richard S. (Department of Pathology, University of Miami, Miami, FL, USA). Journal of Molecular Diagnostics, 2(2), 97-104 (English) 2000. CODEN: JMDIFP. ISSN: 1525-1578. Publisher: Association for Molecular Pathology.

AB The goal of this multicenter study was to evaluate the second-generation Invader technol. for detecting the factor V (Leiden) mutation directly from genomic DNA of different sample types. Invader assay results were compared with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or allele-specific PCR (AS-PCR) anal. The Invader assay is a PCR-independent methodol. that uses a microtiter plate format. In the assay, a specific upstream Invader oligonucleotide and a downstream probe hybridize in tandem to a complementary DNA template and form a partially overlapping structure. The Cleavase VIII enzyme recognizes and cuts this structure to release the 5' flap of the probe. This flap then serves as an Invader oligonucleotide to direct cleavage of a fluorescence resonance energy transfer (FRET) probe in a second **invasive cleavage** reaction. Cleavage of this FRET probe results in the generation of a fluorescent signal. The results of the Invader assay were 99.5% concordant with the PCR-based methods. Of the 372 samples tested once, only two gave discordant results (one from operator error and one from unknown causes), but were concordant on retesting. These results indicate that a simple microtiter plate-based Invader assay can reliably genotype clin. patient samples for the factor V (Leiden) point mutation directly from genomic DNA without prior target amplification.

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1999:164299 Document No. 131:28397 Polymorphism identification and quantitative detection of genomic DNA by **invasive cleavage** of oligonucleotide probes. Lyamichev, Victor; Mast, Andrea L.; Hall, Jeff G.; Prudent, James R.; Kaiser, Michael W.; Takova, Tsetska; Kwiatkowski, Robert W.; Sander, Tamara J.; De Arruda, Monika; Arco, David A.; Neri, Bruce P.; Brow, Marry Ann D. (Third Wave Technologies, Madison, WI, 53719-1256, USA). Nature Biotechnology, 17(3), 292-296 (English) 1999. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature America.

AB Flap endonucleases (FENs) isolated from archaea are shown to recognize and cleave a structure formed when two overlapping oligonucleotides hybridize to a target DNA strand. The downstream oligonucleotide probe is cleaved, and the precise site of cleavage is dependent on the amount of overlap with the upstream oligonucleotide. We have demonstrated that use of thermostable archaeal FENs allows the reaction to be performed at temps. that promote probe turnover without the need for temperature cycling. The resulting amplification of the cleavage signal enables the detection of specific DNA targets at sub-attomole levels within complex mixts. Moreover, we provide evidence that this cleavage is sufficiently specific to enable discrimination of single-base difference and can differentiate homozygotes from heterozygotes in single-copy genes in genomic DNA.

	L #	Hits	Search Text	DBs
1	L1	126	INVASIVE ADJ CLEAVAGE	USPAT ; US-PG PUB
2	L2	185	(NON ADJ TARGET) SAME CLEAVAGE	USPAT ; US-PG PUB
3	L3	83	(NON ADJ TARGET) NEAR4 CLEAVAGE	USPAT ; US-PG PUB
4	L4	41	L1 AND L3	USPAT ; US-PG PUB